

The Occurrence of *Ramphogordius sanguineus* (Nemertea, Heteronemertea) in the Intertidal Zone of the Atlantic Coast of Virginia and New Observations on its Feeding Behavior

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ABSTRACT

The fissiparous heteronemertean *Ramphogordius sanguineus* is common among oysters and mussels on the Rudee Inlet jetty, City of Virginia Beach, Virginia. This is the first report of this species in the intertidal zone of the Atlantic coast of Virginia, but it is likely widespread along the coast. The external morphology, distribution, and life history of *R. sanguineus*, a known predator of polychaete annelids, are reviewed. A description of its feeding behavior is presented, including the novel observation that the proboscis is used to effectively immobilize or slow the locomotion of its polychaete prey (*Alitta succinea*), presumably through toxin delivery.

Key words: external morphology, heteronemertean, intertidal, macrophagous predation.

INTRODUCTION

Nemertean worms are common predators of the marine fauna, feeding on a variety of other invertebrates, including annelids, mollusks, and crustaceans (McDermott & Roe, 1985). Feeding-rates for nemerteans that prey on amphipod crustaceans and those that feed on polychaete annelids suggest that these animals have the potential to impact marine community structure (e.g., McDermott, 1984, 1993; McDermott & Roe, 1985; Ambrose, 1991; Thiel & Kruse, 2001; Caplins & Turbeville, 2011). However, the ecology of nemerteans is understudied relative to many other animal taxa, in part because species identification is often problematic. Twenty-three nemertean species were recorded in intertidal and subtidal waters of Virginia by McCaul (1963) and twenty-four were reported by Wass (1972). Recently, a new record for the state was documented by Turbeville & Caplins (2010). Herein we report the occurrence of the fissiparous heteronemertean *Ramphogordius sanguineus* (Rathke, 1799), in the intertidal zone of the Rudee Inlet jetty, City of Virginia Beach, Virginia. In addition, we present an overview of the external

anatomy, distribution, and life history of this nemertean and provide new laboratory observations on its feeding behavior.

MATERIALS AND METHODS

Nemerteans were collected from the Rudee Inlet jetty, (36°49'49" N, 75°58'06" W) on several occasions during 2009, 2010, and 2011 by removing mussels and oysters (harboring worms) with table knives from the granite rocks, placing them in seawater-filled bags and transporting the bags to the laboratory. Bag contents were then placed in glass culture- or Pyrex baking dishes, and the worms were allowed to crawl away from the mussels and oysters, after which they were transferred to culture dishes containing clean seawater. If worms were observed in the field, they were immediately isolated from the substrate and placed in plastic bags containing seawater. Species identification was based on the description in Coe (1943) and confirmed using divergence and tree-based analyses of mitochondrial DNA cytochrome oxidase I and NADH dehydrogenase 6 gene sequences (unpublished).

For feeding analyses, individuals of the polychaete annelid *Alitta* (formerly *Nereis*) *succinea*, collected as

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above, were placed in a Pyrex dish containing one or more nemerteans, with a cleaned oyster (*Crassostrea virginica*) shell provided as a platform to mimic natural conditions. A volume of water sufficient to cover the shell was added. In some instances, the polychaetes were positioned near the nemerteans using an insect pin or pipette. Observations were made using a Nikon ZMC stereomicroscope. Feeding videos were recorded for subsequent evaluation using a Panasonic DMC-FX37 digital camera mounted on the dissecting scope. Three complete and three incomplete feeding sequences were observed and recorded. Sequences were considered complete if the following events were observed: nemertean proboscis eversion leading to partial or complete prey immobilization followed by prey consumption. Incomplete sequences were analyzed for incidences of nemertean proboscis eversion and prey response. The time from eversion to retraction of the proboscis was recorded and classified according to whether significant contact (e.g., proboscis wrapping around the prey), some contact (e.g., proboscis briefly touching the prey), or no contact was made. Widths of both nemerteans and annelid prey were measured from still images captured from the recorded videos using the program PhotofunStudio (Panasonic), and were made using Image J (Abramoff et al., 2004). Each width measurement represents the average of three measurements taken for each animal from three different still images. Measurements were calibrated from a known distance on the *C. virginica* shell surface.

RESULTS AND DISCUSSION

Habitat, Appearance, and Distribution

Ramphogordius sanguineus, previously known as *Lineus socialis* (see Riser 1994, 1998 for taxonomic revision), occurs commonly on the Rudee Inlet jetty situated among oysters and mussels (*Mytilus edulis*). The worms are often situated in sediment that has accumulated around these sessile molluscs. Other associated fauna include the amphipods *Corophium* cf. *insidiosum*, *Hyale plumulosa*, *Jassa falcata*, the annelid *Allita succinea*, and the nemerteans *Lineus bicolor*, *Prosorhochmus americanus*, *Tubulanus pellucidus*, and *Zygonemertes virescens*.

Individuals vary in color from grayish green to light brown to brownish black or dull red with the posterior regions often appearing lighter in color (Fig. 1). Rudee Inlet jetty specimens are typically brown in color, although some exhibited a dull reddish anterior region. Riser (1994) suggested that diet possibly plays a role in this color variation. This species is characterized in part

by a reddish brain, which is usually apparent in living specimens through the dorsal body wall with incident light (Riser, 1994), deep cephalic grooves on each side of the head, and a row of 3-7 reddish-brown ocelli, situated along each margin of the head (Fig. 2). In a sample of twenty worms, only three individuals exhibited an equal number of ocelli on both margins, an observation congruent with those reported by Riser (1994). Additionally, there are faintly pigmented circumferential constrictions at intervals along the body posterior to the head (Fig. 1). Representative worms collected at Rudee Inlet ranged in length from 30-70 mm and from 0.3-0.6 mm in width, but worms of up to 200 mm long and 1-2 mm wide have been reported in other populations (Coe, 1943; Riser, 1994).

Ramphogordius sanguineus has a global distribution, occurring in the subtidal and intertidal zones of primarily temperate waters. Coe (1943) mentions that along the East Coast of North America, this species occurs from the Bay of Fundy to Florida (without further discussion). This species was not encountered in the surveys of nemerteans of Virginia (Ferguson & Jones, 1949; McCaul, 1963), but is reported in the comprehensive species checklist of Wass (1972). However, because it was reported from subtidal sands (an atypical habitat) of the York River and no description is included, this record is questionable. Given that *R. sanguineus* is abundant on hard substrates in the intertidal zone north and south of Virginia, its discovery on the Rudee Inlet jetty was expected, and it was likely missed in past surveys because of its patchy distribution (pers. obs.) and sampling strategies utilized. Our observations thus verify the presence of this species in Virginia, and suggest that it is likely abundant in other fouling communities along the coast.

Besides its common association with mussels and oysters on rocky shores (Riser, 1993, 1994) and artificial hard-substrate (e.g., jetties, groins, pilings; Fox & Ruppert, 1985; pers. obs.) communities, this species also occurs in algae, beneath stones on fine sediment, or in fine sediment (Gibson, 1995, 2001) and, as pointed out by Riser (1994), Verrill (1873) lists *R. sanguineus* as a nemertean that occurs on submerged wood, buoys, and boat bottoms. This species tends to be gregarious and may be found in large numbers at a given location. It can occur syntopically with *Lineus ruber* and *Lineus viridis* in the northern reaches of its range in North America (Coe, 1943; J. Norenburg, pers. comm.), and because of overlapping color variation, it sometimes may be confused with the latter species. *Ramphogordius sanguineus* can be distinguished from *L. ruber* and *L. viridis* by its habit of coiling irregularly

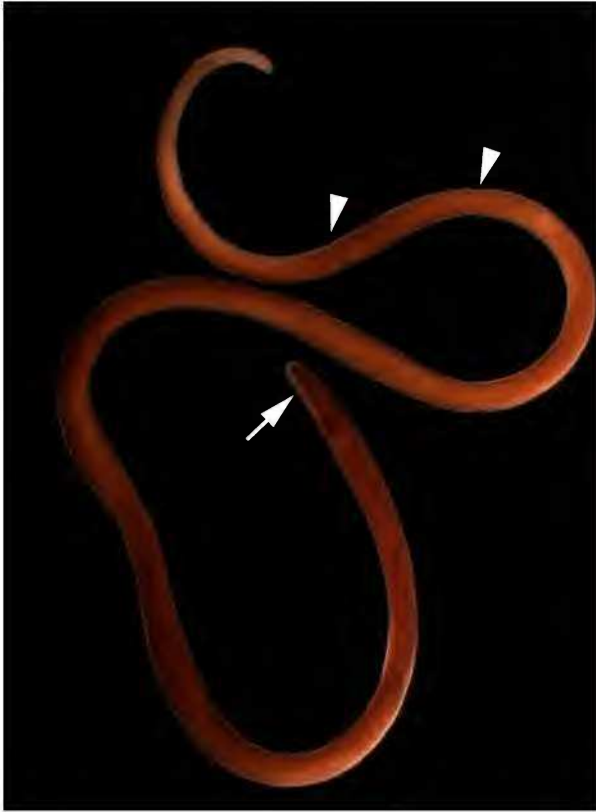


Fig. 1. Dorsal view of *Ramphogordius sanguineus*. The left cephalic groove (arrow) and some of the circumferential constrictions (arrowheads) are apparent.

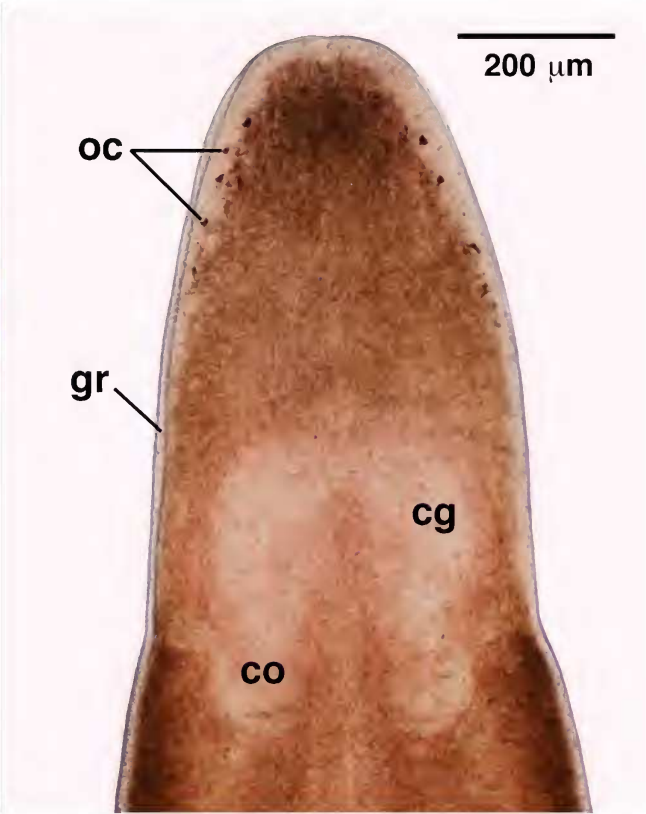


Fig. 2. Light micrograph of the anterior end of *Ramphogordius sanguineus* revealing the ocelli (oc), cerebral ganglia (cg), cerebral organs (co), and the left cephalic groove (gr).

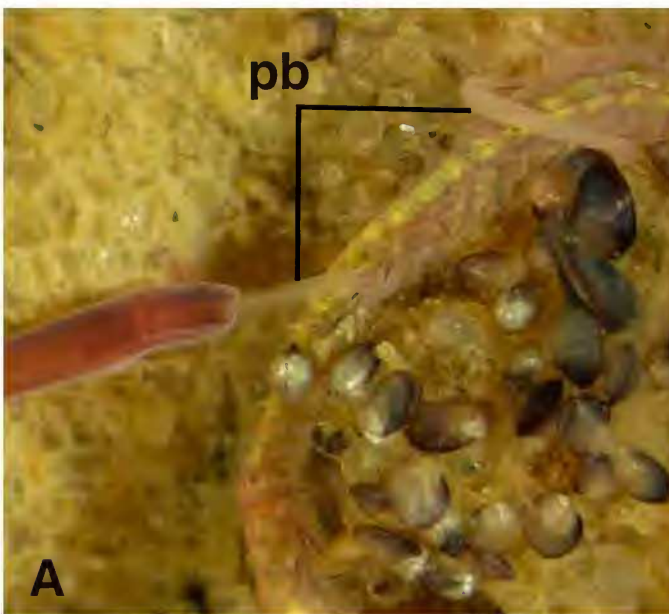


Fig. 3. Snapshots from videos of *Ramphogordius sanguineus* feeding on *Alitta succinea*. A) Image showing the everted proboscis (pb) coiled loosely around *A. succinea*. Note juvenile mussels. B) *R. sanguineus* engulfing *A. succinea* such that it forms a J-shape while another nemertean approaches.

or into a spiral when disturbed. This coiling behavior is considered diagnostic (Coe, 1943; Gibson, 2001; J. Norenburg, pers. comm.).

Ramphogordius sanguineus reproduces asexually (clonally) by fragmentation, and the previously mentioned circumferential constrictions in many cases correspond to the fission zones, although fission can occur outside these regions (Coe, 1930). Each fragment, which may encyst in mucus, develops into a complete worm by anterior and posterior regeneration (Coe, 1930). Fissiparity also distinguishes *R. sanguineus* from *L. ruber* and *L. viridis*, which are incapable of asexual reproduction and exhibit anterior regeneration only anterior to the brain (Coe, 1943).

Widespread distribution of *R. sanguineus* may be attributable to rafting on debris or transport on boat bottoms (Riser, 1993, 1994), a common mechanism of dispersal of fouling organisms (see Highsmith, 1985; Thiel & Gutow, 2005a, b). It is also possible that encysted worms, which can remain in this state for weeks or months (Coe, 1943), could be dislodged from the substrate and dispersed by water currents. Accounts of sexual reproduction in this species are fragmentary, and whether a free-swimming larval stage exists is unknown (see Coe, 1943; Riser, 1994).

Feeding Observations

Jennings & Gibson (1969) described the basic feeding mechanism of *R. sanguineus*, and many of our observations are consistent with theirs, but we provide additional details and highlight some notable differences. This species is a macrophagous predator of polychaete annelids, although when starved, it will consume oligochaetes and sometimes other nemertean species (Jennings & Gibson, 1969). In our observations, predation began with the nemertean moving toward the polychaete prey (*Allita succinea*), presumably following chemical cues (see Jennings & Gibson 1969; Roe, 1970), and then everting its proboscis, which would often coil loosely around the annelid when contact was made (Fig. 3A). Proboscis eversion was elicited either by the nemertean touching the annelid with its head and pulling back slightly before everting its proboscis, or when the annelid was within striking distance, 0.5-0.6 mm ($n = 2$ observations). The striking distance appeared to be greater in other instances, but for these we were unable to obtain measurements. Also, in these cases, the proboscis typically did not make contact with the annelid. The annelid's response to a proboscis strike was somewhat variable: in one instance, the annelid struggled wildly by twisting and coiling before it was effectively immobilized (lying on

its dorsal surface, slowly twitching and everting its pharynx slightly) following brief contact (5 s) with the proboscis. It remained immobilized in this manner for approximately 3 min before the nemertean returned to consume the annelid whole (<http://www.vimeo.com/13829966>), which took approximately 80 s. In another case, the annelid appeared distressed, twisting and coiling following contact (7 s) with the nemertean proboscis, but did not show the same degree of immobilization as mentioned above, and instead appeared slightly stunned or disoriented. The annelid recovered in 10 seconds but was attacked again and consumed after 1 min.

Additionally, in several cases we observed the nemertean everting its proboscis after it had attached its mouth to the annelid. In an observation involving two nemerteans, one everted its proboscis five times and the other three times as they attempted to swallow the still struggling annelid, which they succeeded in immobilizing and consuming. These preliminary observations suggest that the proboscis of *R. sanguineus* delivers toxins to immobilize or at least partially slow its prey, in contrast to the claim of Jennings & Gibson (1969) that it is used only to wrap tightly around and pull the annelid towards the mouth of the nemertean. We observed only two cases in which the proboscis may have played a minor role in gripping the prey, making brief contact (4 s and 3 s, respectively) before the annelid was released. The proboscis is equipped with secretory cells that produce rod-shaped structures, termed pseudocnidae (see Turbeville, 2006) that could serve to grip and possibly puncture prey (Jennings & Gibson, 1969), thus allowing toxin entry, but we were unable to document their role in the capture or immobilization of *A. succinea*.

In six separate feeding observations involving a total of 14 nemerteans and six annelids, 32 instances of nemertean proboscis eversion occurred. Half (16) of these eversions made significant, although somewhat loose, contact with the prey item (coiling or wrapping around it), and the proboscis was fully retracted after an average of 5.3 s (range = 2-16 s) of contact with the annelid prey. The remaining proboscis eversions either made no contact ($n = 10$, mean = 2.2 s, range = 1.5-3 s) or made some contact ($n = 6$, mean = 3.3 s, range = 3-4 s). These data support our observations that proboscis contact with prey is typically relatively brief and is likely the primary means of immobilizing prey through toxin delivery. However, it is unclear from our limited number of observations whether a correlation exists between the degree of annelid immobilization and the duration of contact with the proboscis.

Concurrently, or shortly following proboscis

eversion, the mouth of the nemertean (subterminal to the head/proboscis pore) dilates as it crawls towards its annelid prey. Once the mouth makes contact with the prey, peristaltic waves of the body-wall musculature, along with mucous secretions from gland cells located in the buccal cavity and foregut (see Jennings & Gibson, 1969), aid in the engulfment of the immobilized or partially immobilized annelid (Fig. 3B). Occasionally, the head appeared to be pressed against the prey and used to hold and guide the annelid into the nemertean's mouth, but during other predation events was raised above the annelid as the mouth dilated and when the annelid was engulfed (Fig. 3B; see Jennings & Gibson, 1969; Wang et al., 2008). *Ramphogordius sanguineus* does not appear to have a preference for the orientation of the prey item preceding consumption; we observed annelids being consumed linearly, either head-first, or tail-first, as well as folded over into a V or J shape, depending on where along the annelid body the nemertean began to engulf it (Fig. 3B).

The consumption time of two predation events, both involving five nemerteans attacking one annelid, was 4 min 50 s and 3 min 25 s (width measurements were unattainable for these events). For predation events involving one nemertean, the consumption times are as follows: 1 min 20 s for a nemertean of 0.632 mm (SD = 0.02) in width and an annelid 0.676 mm (SD = 0.03) in width, and 50 s for a nemertean 0.52 mm (SD = 0.05) wide that consumed about 1/3 of the posterior end of an annelid 0.56 mm (SD = 0.08) wide, torn off against the sharp edge of an oyster shell. These consumption rates are within the range documented for the palaeonemertean *Procephalothrix simulus* consuming the polychaete *Saccocirrus gabrillae*, that had been partially or completely immobilized by the nemertean's proboscidal toxins (Wang et al., 2008), and are broadly consistent with Roe's (1970) observations concerning macrophagous predation by the hoplonemertean *Paranemertes peregrina* on polychaetes when the prey item was less than the diameter of the dilated mouth of the nemertean.

We made three observations involving multiple nemerteans feeding on one annelid. These events were similar to those involving one nemertean, but with some notable behavioral differences. Namely, each nemertean that approached the site of predation exhibited proboscis eversion, regardless of the annelid's state of immobilization. Additionally, the nemerteans were each able to consume some portion of the annelid by pinching off pieces of the annelid, a process likely aided by the release of digestive enzymes and the muscular contractions of each nemertean's mouth and foregut (<http://www.vimeo.com/24638715>; see

Jennings & Gibson, 1969).

Throughout our observations of predation events, the annelid prey (*Allita succinea*) exhibited putative anti-predatory responses when in close proximity to the nemerteans, including agitation, rapid crawling, swimming, and pharynx eversion. In one case the annelid secreted mucus in a trail behind it perhaps to facilitate escape or slow the advance of the nemertean, and in other cases a swimming response was elicited and were it not for the confines of the dish, and our return of the annelid back to the feeding arena, the annelid likely would have escaped predation. Controlled experiments will be necessary to verify if the observed behaviors represent escape responses.

Our qualitative observations suggest that *A. succinea* is the most abundant errant polychaete in the jetty fouling community, but whether it is the favored prey of *R. sanguineus* will remain unknown until feeding preference studies are conducted.

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